ACUTE INHALATION TOXICITY OF SAXITOXIN TO MICE

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Systemic toxicity of saxitoxin (STX) in a variety of laboratory animals is well documented. Reports of toxicity from respiratory exposure, however, are negligible. In this study a concentration-response curve was determined for mice exposed to aerosols generated from a saline solution of STX. For a 10 min exposure, the STX aerosol concentration calculated to kill 50% (LC_{50}) of the exposed mice was ~0.3 μg STX/liter air. Results from aerosol deposition studies in mice exposed to an LC_{50} concentration of STX aerosol gave an LD_{50} of 0.9 μg/kg. Thus, STX was at least 10 times more toxic to mice by aerosol exposure than by systemic administration (LD_{50} - 10.0 μg/kg; i.v., i.p.). The mechanism of this enhanced toxicity is presently unknown.

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D.A. CREASIA and M.L. NEALLY. Acute inhalation toxicity of saxitoxin to mice. *Toxicon* , , , 19 . Systemic toxicity of saxitoxin (STX) in a variety of laboratory animals is well documented. Reports of toxicity from respiratory exposure, however, are negligible. In this study a concentration-response curve was determined for mice exposed to aerosols generated from a saline solution of STX. For a 10 min exposure, the STX aerosol concentration calculated to kill 50% (LC_{50}) of the exposed mice was (approx) (micrograms) ~0.3 μg STX/liter air. Results from aerosol deposition studies in mice exposed to an LC_{50} concentration of STX aerosol gave an LD_{50} of 0.9 (micrograms) μg/kg. Thus, STX was at least 10 times more toxic to mice by aerosol exposure than by systemic administration (LD_{50} ~ 10.0 μg/kg; i.v., i.p.). The mechanism of this enhanced toxicity is presently unknown. (AW)

The views of the author(s) do not purport to reflect the positions of the Department of the Army or the Department of Defense.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of laboratory Animal Resources Commission on Life Sciences-National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

INTRODUCTION

Saxitoxin, (STX), one of the most lethal non-protein toxins known (DAVIO, 1985) is produced by dinoflagellates of the genus *Gonyaulax* (SOMMER *et al.*, 1937, SHANTZ *et al.*, 1966). *Gonyaulax* dinoflagellates can contaminate shellfish, which, when eaten by humans have been known to cause numbness, paralysis and death due to respiratory arrest. The toxicity of systemically administered STX is well documented (DAVIO, 1985, SCHANTZ, 1986, SHIMIZU, 1986). The paralysis and respiratory arrest observed following ingestion of STX results from STX reducing the permeability of the sodium channel of nerve and muscle membranes. It is feasible that inhaled STX might produce respiratory paralysis through direct action on sodium channels of respiratory tract nerve and muscle tissue. However, there are few, if any, well-documented studies that report on results from respiratory tract exposure to STX. The data reported here were derived from a series of experiments designed to determine the relative potency of inhaled STX as compared to systemically administered STX.

MATERIALS AND METHODS

Animals

Male VAF/Plus, CD-1, five-week-old mice weighing 20-23 g each were obtained from Charles River Labs, Inc. (Wilmington, MA). All mice were held five per open-bottom polycarbonate cage with free access to food (NIH formula 07) and water. The mice were held in rooms maintained at 24°C and 50% relative humidity and air flow was 12 room air changes per hr. All mice were held 1 week for acclimatization and observation prior to being used in the study. The average weight of each mouse was 25 g when used.

Chemicals

STX used in this study was high-performance-liquid-chromatography (HPLC) purified (HALL and REICHARDT, 1984) and obtained from Dr. Sherwood Hall of the Food and Drug Administration (Washington, D.C.). Synthetic, non-exchangeable $[^3H]$ saxitoxinol (specific activity, 18-75 Ci/mmole) was obtained from Dr. F.S. Chu (University of Wisconsin, Madison, WI) for use as a tracer.

Aerosol Exposure

The inhalation exposure chamber is a 1.5 liter rectangular box with six ports on either side for nose-only exposure. The mice were exposed to the aerosol by placing each mouse in an open-ended, conical holder with only its nose protruding into the aerosol chamber. The chamber was operated dynamically at 2.2 liters air/min. Test aerosols were generated by atomizing a saline solution of STX with a Lovelace nebulizer (Intox Products, Alburquerque, NM) operated at 2.2 liters air/min. (MERCER *et al.*, 1968). The saline solutions of STX were prepared by first dissolving weighed quantities

of crystalline STX in measured volumes of physiological saline. Fifty microliters of [^{3}H]saxitoxinol in saline was added as a tracer for aerosol mass-concentration measurements, and 0.5 ml of the same solution was substituted for 0.5 ml of physiological saline for deposition and retention studies in mice exposed to the STX aerosol. The mass concentration of the STX aerosol was varied by atomizing different concentrations of the STX solution and was measured by scintillation counting (Beckman L55 800 scintillation counter, Beckman Instruments, Inc., Irvine, CA) of the [^{3}H]saxitoxinol tracer. Samples for aerosol mass-concentration measurements were obtained from 1.0 liter samples taken on fiberglass filters (Gelman Scientific Inc., Ann Arbor, MI). The range of aerosol mass concentration was 0.5 to 20 µg STX/liter air. Aerosol particle size was determined for each aerosol mass concentration with a Mercer-type (MERCER *et al.*, 1962), cascade impactor (Intox Products, Alburquerque, NM) operated at 100 cm^3 air/min. The average aerodynamic mass median diameter of the aerosol particles was 0.6 µm with a σ g of 1.6 to 1.8. The aerosol remained as spherical liquid droplets as observed under the light microscope, indicating that the STX aerosol in this study was actually an aerosol of a saline solution of STX. All aerosol exposures were for 10 min. After exposure to the STX aerosol, animals were observed at least twice daily for 1 week post-exposure. The LD_{50} value was calculated by probit analysis of the slope of the concentration-response lines (Finny, 1977). Data used to calculate the 24 hr LC_{50} are based only on those animals that died within 24 hr post-exposure.

Retention of inhaled STX

Retention of inhaled STX was measured in two groups of 12 mice each. One group was exposed to a STX aerosol with a mass concentration of 0.95 µg

STX/liter air and the other group was exposed to an aerosol with a mass concentration of 0.38 μg STX/liter air (~LC_{50}). Both groups were arbitrarily subdivided after aerosol exposure into two groups of six mice each; one of which was observed for lethality for 24 hr post-exposure and the other which was killed by CO_2 asphyxiation immediately (i.e. within a few min post-exposure). The intact carcasses of six of the mice killed by CO_2 asphyxiation were each placed separately into 50 ml of 2N KOH and incubated overnight at 37°C. Triplicate 1.0 ml aliquots were then taken and assayed for [^{3}H].

Systemic Administration of STX

STX was administered to mice by both i.p. and i.v. (tail vein) injection according to standard laboratory procedures. All injection volumes were 0.1 ml. The STX used in this aspect of the study was obtained from aliquots taken prior to nebulization from the STX preparation used for aerosol generation.

RESULTS

Data comparing the lethal effects of groups of mice exposed for 10 minutes to various concentrations of STX aerosol are summarized in Table 1. At aerosol concentrations ≥ 1.6 μg/liter STX, all mice died prior to termination of the aerosol exposure. One mouse from the group of mice exposed to 1.0 μg/liter air had survived the 10 min aerosol exposure but was gasping when removed form the aerosol chamber and died within a few minutes post-exposure. None of the mice which survived exposure to lower concentrations (i.e. ≤ 0.5 μg/liter) of STX aerosol showed any gross signs of toxicity and, when removed from the holders, immediately began grooming themselves and/or exploring their cage. None of the mice that survived the STX aerosol exposure died within the 1 week post-exposure observation period.

Retention of Inhaled STX

These data are summarized in Table 2. From the group of mice exposed to 0.95 μg STX/liter air six of six mice arbitrarily selected for observation died, indicating that the aerosol mass concentration was in the lethal range. In the group of mice exposed to 0.38 μg STX/liter air, four of six mice died, indicating an aerosol mass concentration in the LC_{50} range.

Systemic Administration of STX

The LD_{50} of STX for mice injected i.p. was 11.7 μg STX/kg body weight (9.0-1.6.3, 95% C.I.), and 9.6 μg STX/kg body weight (7.6-12.4, 95% C.I.) by the i.v. route. These data compare faborably with that already published (DAVIO, 1985).

DISCUSSION

In this study, mice were exposed to various aerosol mass concentrations of STX and a concentration-response relationship was developed. From these data, we determined that the LC_{50} for mice exposed to a STX aerosol was 0.3 µg, STX/liter air. In mice exposed to an LC_{50} STX aerosol concentration, we found that the total retained dose of inhaled STX was 0.023 µg STX per mouse. Thus, for the 25 g mice used in this study, the LD_{50} of inhaled STX was approximately 0.9 µg STX/kg body weight, which was approximately 10 times more toxic than with STX administered i.p. or i.v.

We have not explained the increased toxicity of inhaled STX compared to systemically administered STX. Usually, enhanced toxicity from inhaled chemicals is associated with pulmonary damage, subsequent pulmonary edema and hemorrhage; death usually results from asphyxiation from impaired gas (O_2, CO_2) exchange. Pulmonary pathology sufficient to produce impaired gas exchange is readily apparent even under gross examination. However, mice dying from inhaled STX, even at the highest concentrations used in this study did not exhibit any gross pulmonary pathological changes when the lungs were examined, essentially ruling out death from impaired gas exchanged.

It is possible that inhaled STX may act through unknown mechanism(s) associated with the lung. Studies designed to address this question are currently underway.

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TABLE 1. MOUSE MORTALITY FROM INHALED SAXITOXIN*

AEROSOL CONC. (μg/liter air)	NO. DEAD/ NO. EXPOSED	TIME TO DEATH (MIN)
20	6/6	<10
10	6/6	<10
5	6/6	<10
2.2	6/6	<10
1.6	6/6	<10
1.0	6/6	12-15
0.5	3/6	<10
0.15	1/6	<10
0.1	2/6	<10
0.05	0/6	--

24 hr LC_{50} ~ 0.3 μg STX/liter air (0.16 - 0.48, 95% C.I.)

*A 10-min aerosol exposure.

TABLE 2. RETENTION OF INHALED SAXITOXIN IN MICE*

AEROSOL CONC. (μg/liter air)	NO. DEAD/ NO. EXPOSED	RETAINED SAXITOXIN† (μg equivalent)‡	SAXITOXIN DOSE (μg/kg body weight)
0.95	6/6	0.145 ± 0.05	5.8 ± 2.0
0.38	4/6	0.023 ± 0.016	0.92 ± 0.5

*A 10-min aerosol exposure.

†N = 6;

‡Mean ± S.E.

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